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ABSTRACTS OF PAPERS PRESENTED AT THE ANNUAL MEETING, EDMONTON, ALBERTA, JANUARY 16, 1963

1. THE RELATIONSHIP BETWEEN FATTY ACID STRUCTURE AND THE RELEASE AND ESTERIFICATION OF FREE FATTY ACIDS (FFA) IN RAT ADIPOSE TISSUE

A. Angel and C. H. Hollenberg, McGill University Medical Clinic, Montreal General Hospital, Montreal.*

Incubation of rat adipose tissue with corticotropin in buffer-albumin results in accumulation of FFA within the tissue and release of the acids into the medium. Gas chromatographic analysis of tissue and medium FFA revealed proportionately more myristic, palmitoleic, linoleic and linolenic acids in the medium. The ratio of the amount of each FFA in the medium to that in the tissue was calculated and was found to increase progressively with both decreasing chain length and increasing unsaturation.

The esterification rate of FFA in rat adipose tissue was also found to be related to fatty acid structure. FFA production in this tissue was stimulated by incubation with corticotropin, following which esterification in half the specimens was enhanced by exposure to glucose and insulin. Determination of the compositions of the FFA in the control and glucose/insulin tissues revealed that amongst the saturated and monoenoic acids the shorter chain components were esterified to a greater extent, while at a given chain length the more unsaturated acids were more readily esterified.

The data indicate that the more polar FFA were more readily released from and esterified in adipose tissue. This may be due to greater solubility of these acids in aqueous medium or cell cytoplasm where esterification occurs. It is possible that adipose tissue contains free acid binding sites, and if affinity to these sites is similar to that described for albumin, the shorter chain and more unsaturated free acids would be less firmly bound and more available for release or esterification.

2. FAMILIAL ELECTROLYTE-LOSING NEPHROPATHY

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Seven-month-old twin female infants were admitted to hospital with failure to thrive, polyuria and dehydration. Laboratory investigation revealed a severe degree of alkalosis, hypokalemia, hypochloremia and hyponatremia. Serum calcium and inorganic phosphate levels were also low. Twenty-four hour excretion of aldosterone (10 and 28 μg.) was within the normal adult range. Attempts at complete correction of dehydration, alkalosis and electrolyte deficits were unsuccessful because of excess losses in the urine. Intravenous therapy with sodium, potassium, chloride, calcium and fluid failed to prevent neurological complications with deterioration of mental status, lethargy, convulsions and spasticity.

Further investigation showed extremely low levels of serum magnesium—0.77 to 0.79 mEq./l. (normal 1.40 to 1.88 mEq./l.). Eight milliequivalents of magnesium, given intravenously daily over five hours, resulted in improved clinical status with increased activity and weight gain. Other electrolytes could also be maintained more easily with oral therapy.

A sibling of these twins had died eight years previously with similar biochemical abnormalities. Autopsy studies on this patient and another infant who died with a similar picture showed a prominent nephrogenic zone with deeper hypertrophic glomeruli and vacuolar nephropathy. Juxtaglomerular cells showed striking hypertrophy and hyperplasia. In the gross, the adrenals were small but histologically the zona glomerulosa was wide relative to the zona fasciculata.

3. THE USE OF MANNITOL DURING SURGERY OF THE ABDOMINAL AORTA

R. J. Baird, W. S. Firor and H. W. K. Barr (introduced by R. W. Gunton), Department of Surgery, Toronto General Hospital, Toronto, Ont.*

Five of 28 patients who underwent operations in 1960-61 that required cross-clamping of the abdominal aorta, developed postoperative renal failure and died. The operative and postoperative urinary output of the next 27 patients was carefully studied. Fourteen patients received intravenous mannitol infusions before and during the period of aortic occlusion; the other 13 received an equivalent volume of dextrose and water. No instance of renal failure occurred in either group. The urinary output of the patients who received mannitol was markedly greater than that of the others.

As well as demonstrating the value of mannitol, this study emphasizes the importance of adequate hydration during this type of surgery. We believe that in addition to its demonstrated effectiveness,

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the simplicity and safety of a mannitol infusion is such that its use is indicated in operations involving cross-clamping of the abdominal aorta. It is particularly indicated during the resection of the ruptured abdominal aortic aneurysm.

4. ORIGIN OF "BURR" ERYTHROCYTES

R. Edward Bell, University Hospital, Edmonton, Alta.

The term "burr" cell has been used to describe irregularly shaped erythrocytes with one or more large projections along the periphery of the cell. They are found most frequently in bleeding peptic ulcer, gastric carcinoma and uremia, and less frequently in hemolytic anemias in association with fragmented erythrocytes (schistocytes), triangular forms or other unusual shapes. No satisfactory theory has been advanced to explain the origin or development of these cells. Evidence has been produced that mechanical effects may be a major factor, but unknown metabolic defects or "toxic" causes may be important.

Morphological evidence is presented from observations made on erythrocytes in blood films of patients with these cells that suggests a probable mechanism of their production. The sequence of events terminating in production of burr cells appeared to be the development of a vacuole or blister at the cell surface which enlarges and ruptures, leaving a distorted erythrocyte with thin projections from the shoulder of the crater left by the eruption of the vacuole. Further distortion of the erythrocyte results in the typical burr cell. The implications of this theory on formation of other abnormally shaped erythrocytes and on hemolytic disease are discussed.

5. EXPERIENCE WITH THE TERRY LIVER BIOPSY NEEDLE

Alan F. Bond and Neville J. Jackson, Cancer Clinic, University Hospital, and Department of Surgery, University of Saskatchewan, Saskatoon, Sask.

The Terry biopsy needle was originally devised by Dr. Terry of St. Bartholomew's Hospital, London, for performing liver biopsies. It is, however, a very useful and versatile instrument which may be used for needle biopsy purposes in many instances other than liver biopsy.

Its main advantages are that: (a) a large core of tissue may be obtained so that the general architectural pattern, in addition to the cellular details, may be seen; (b) the needle is easy to use and is effective in a very high proportion of cases.

Needle biopsy has been freely used in a wide variety of liver diseases including portal and biliary cirrhosis and primary and metastatic malignant disease of the liver. The needle has also been used quite extensively to obtain biopsies of various tumours, either superficial or deep-seated, elsewhere in the body. More than 70 biopsies have been performed.

The employment of the Terry biopsy needle for renal biopsy is not recommended.

6. PULMONARY DIFFUSING CAPACITY IN POLYCYTHEMIA VERA

J. H. Burgess and J. M. Bishop (introduced by C. H. Hollenberg), Department of Medicine, Queen Elizabeth Hospital, University of Birmingham, Birmingham, England.

Pulmonary diffusing capacity for carbon monoxide (DL), membrane diffusing capacity (Dm), and pulmonary capillary blood volume (Vc) were measured in 10 patients with polycythemia vera: (a) before treatment, (b) after venesection one week later, and (c) three months after treatment with P^{32}.

Before treatment DL was higher than the predicted normal in most patients, apparently owing to the increased hemoglobin concentration in the pulmonary capillaries. DL was decreased after treatment in all patients. Dm showed no significant change with treatment. Vc appeared to decrease with treatment, corresponding with a fall in measured total circulating blood volume. DL was below the predicted normal in three patients after treatment.

7. INOTROPIC EFFECT OF L-NORADRENALINE IN EXPERIMENTAL CARDIOGENIC SHOCK

R. F. P. Cronin and E. H. Tan, McGill University Medical Clinic, Montreal General Hospital, Montreal, P.Q.*

Hemodynamic effects of L-noradrenaline infusion (NE) were studied in 10 dogs. Each animal was anesthetized with pentobarbital and 10 ml./kg. of blood was withdrawn, the drawn blood being replaced by an equal amount of dextran solution. Thirty minutes later, closed chest coronary embolization was carried out in five dogs, which resulted in the production of cardiogenic shock as judged by a 30% or greater reduction in mean arterial blood pressure (MBP) and a 50% or greater reduction in cardiac output (CO). The remaining five animals acted as controls.

MBP was obtained by catheterization of the aorta and left ventricular mean filling pressure (MFP) by trans-septal catheterization of the left heart. CO was derived from directly inscribed dilution curves utilizing T-1824 dye and a densitometer-recorder system. Left ventricular stroke work (LVSW) was calculated from the foregoing data.

CO, MBP and MFP values were studied for 30 minutes to ensure a steady state. NE was then infused at a constant rate of 1 μg./kg./min. for 15 minutes and the observations were repeated.

Fifteen minutes after discontinuing NE, reinfusion of the previously drawn blood was started and continued until the MFP had risen to the value observed during NE infusion.

In the control group, NE infusion resulted in an average increase in left ventricular stroke work of 91% for a rise of 6 mm. Hg in mean filling pressure. Transfusion in the control animals caused a 72% rise in LVSW for the same rise in MFP.

In the shocked animals, NE infusion increased LVSW by 120%, but autologous transfusion caused only a 40% rise for the same increase in MFP.

These data indicate that the inotropic effect of infused L-noradrenaline is enhanced in experimental cardiogenic shock.

8. BLOOD COAGULATION AND PLATELET ECONOMY IN SUBJECTS WITH MYELOPROLIFERATIVE DISORDERS

J. H. Crookston, E. A. Murphy, J. G. Scott and J. F. Mustard, Department of Medicine, University of Toronto, Toronto General Hospital and Sunnybrook Hospital, Toronto, Ont.*

Platelet survival and turnover were studied by means of DFP^{32} in 33 subjects with myeloproliferative disorders. The mean platelet half-life value was not significantly different from that found in 44 control subjects. Mean platelet turnover, however, was more than doubled.

Since the platelet survival values were spread over a wide range, the data were examined in relation to the presence of thrombotic or hemorrhagic complications. Patients with thrombotic complications had a shorter mean platelet survival than the controls, while patients with hemorrhagic complications had a longer mean platelet survival value. Patients with a normal platelet count but short platelet survival had platelet turnover values comparable to those found in patients with high platelet counts. A significant positive correlation was found between the platelet count and platelet survival values.

The patients with normal platelet counts and short platelet survival values did not show coagulation defects, while patients with high platelet counts did show abnormalities, chiefly in the prothrombin time and thromboplastin generation test. The prothrombin time defect was related to low levels of factor VII and factor V activity. The thromboplastin generation test showed defects in the $Al(OH)_3$-absorbed plasma and in the serum fractions. There were also, at times, platelets which functioned poorly in the test system. The abnormal serum would not correct serum deficient in factor IX, when phospholipid was used as the lipid source in the thromboplastin generation test. However, when platelets were used, a factor IX defect could not be shown. Furthermore, the abnormal serum could be corrected by the addition of trace amounts of thrombin which would not correct factor IX deficient serum. The serum defect seems to be related in part to antithrombin or antithromboplastin activity. The $Al(OH)_3$-plasma defect appeared to reflect a reduction of factor VIII activity. This defect may relate to absorption of factor VIII on to platelets, since a similar defect can be produced *in vitro* by adding high concentrations of hemophilia A platelets to normal plasma.

When the platelet count becomes normal after therapy with P^{32} or busulfan, the clotting defects disappear. It would appear that a high platelet count, but not necessarily a high platelet turnover, can be associated with disorders of clotting.

9. THE TEMPORAL RELATIONSHIP OF THE OXYGEN DEBT TO TREADMILL EXERCISE

T. E. Cuddy, P. Caldini, D. N. Gupta and R. M. Cherniack,* Department of Medicine, University of Manitoba, Winnipeg, Man.*

Oxygen debt has been interpreted as an expression of anaerobic metabolism. It has been generally accepted from measurements of "excess lactate" that the oxygen debt develops at a constant rate during exercise, the amount varying directly with the duration and severity of the muscular work.

To elucidate the rate of development of the oxygen debt, three male subjects were studied on 10-15 occasions while walking on a treadmill at three miles per hour at different slopes for periods of time varying between one and 32 minutes. During each period of exercise, oxygen consumption, minute ventilation and oxygen debt were calculated, employing open-circuit techniques. The order of the exercise was randomly selected.

In all three subjects, there were two "phases" of oxygen debt. A relatively high debt (73-440 ml./minute) was present at the end of a one-minute exercise and, following this, as the duration of exercise was increased, the oxygen debt rose at a constant rate (4.7-8.4 ml./minute) over the next 30 minutes. Both the early and late phases were elevated by increased severity of exercise (increased treadmill slope).

In order to determine whether the early oxygen debt could be due to extraction of oxygen from the body stores, the study was repeated in one subject when the body stores of oxygen were reduced by breathing 10.0, 15.0 and 20.9% O_2 in N_2. The early phase was still present and was increased by diminishing O_2 concentrations. This suggested that the O_2 debt developed early in exercise and was not due solely to extraction of body stores of oxygen.

10. L-NORADRENALINE EFFECT ON RIGHT ATRIAL PRESSURE AND THE SQUARE WAVE RESPONSE TO VALSALVA'S MANEUVER

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Right atrial pressures were measured in 22 patients before and immediately after the cessation of airway straining. In nine patients (Group 1) showing a normal peripheral arterial blood pressure response to the Valsalva maneuver the resting mean right atrial pressure of 6.7/2.7 mm. Hg rose to a mean of 12.2/7.6 mm. Hg ($p = <0.01$) during phase 4 of the overshoot. Thirteen patients (Group 2), including six with congestive heart failure, responded with the square wave phenomenon to Valsalva's maneuver. The resting mean right atrial pressure of 16.5/11.6 mm. Hg remained unchanged after cessation of airway straining ($p = >0.1$) and differed significantly from that of Group 1 ($p = <0.01$).

Infusion of L-noradrenaline (4 mg./l.) into the right atrium of the nine patients of Group 1 led to the emergence of a square wave response to the Valsalva maneuver within approximately 10 minutes of the infusion and subsequent to the maximum elevation in peripheral arterial blood pressure. The resting mean right atrial pressure rose significantly from 6.7/2.7 mm. Hg to 15.8/10.3 mm. Hg ($p = <0.01$) and remained unchanged after the Valsalva maneuver, i.e. 15.7/10.1 ($p = >0.1$). No statistical difference obtained between the ambient mean right atrial pressures of Group 2 and the L-noradrenaline infused patients ($p = >0.1$), and it is suggested that an elevated central venous and right atrial mean pressure of approximately 13 mm. Hg in the presence of an increment of intrathoracic pressure not exceeding 40 mm. Hg becomes associated with the square wave phenomenon.

11. THE EFFECT OF RADIOPHOSPHORUS (P^{32}) ON POLYCYTHEMIA RUBRA VERA

H. E. Duggan and Dorothy J. Weijer (introduced by D. R. Wilson), Department of Medicine, University of Alberta, Edmonton, Alta.*

The concept of selective uptake of P^{32} by the hematopoietic tissues of the body is reviewed. Evidence against a high selective uptake is to be found in the existing literature and is provided by studies based on assay of radioactive autopsy specimens (and autoradiographs) from a deceased polycythemic patient. It is proposed that the mechanisms underlying the response of polycythemia rubra vera to therapy with P^{32} consist of: (a) some degree of ionization, but chiefly (b) incorporation of P^{32} into the genetic material of the stem cells. This P^{32} subsequently decays to S^{32}, causing breaks in the D.N.A. and a subsequent reduction in the number of circulating erythrocytes.

12. METABOLISM OF CORTICOSTEROIDS BY INCUBATED LIVER SLICES

Inge Dyrenfurth and Lionel McLeod,* Department of Medicine, University Hospital, Edmonton, Alta.*

Human and rat liver slices were incubated in Krebs-Ringer solution with cortisol and corticosterone as substrates without the addition of cofactors. The incubation mixtures were extracted for the hormone metabolites, which were then separated by paper chromatography. Unchanged substrate, 11-oxidized and ring-A reduced metabolites were determined.

Samples of 11 human livers obtained at autopsy, five to 11 hours post mortem, were incubated with cortisol. All tissues were found to be metabolically active. Samples of four normal livers showed 20-45% unchanged cortisol after three hours of incubation. Cortisone formed was from 2-8%, tetrahydrocortisone (THE) 1-4%, tetrahydrocortisol (THF) 1-7%, and dihydrocortisone (DHE) 1-5%. Samples of a fatty liver did not form THE, THF or DHE, though cortisol disappeared rapidly and much cortisone (13%) was formed. Two experiments with congested livers showed a delayed disappearance of cortisol, somewhat increased DHE (7 and 10%) and greatly increased cortisone (22 and 24%). The ring-A reduced metabolites were present in normal amounts. Tissue from a cirrhotic liver also demonstrated a slow disappearance of cortisol and larger amounts of cortisone (18%). Samples of a liver containing cancerous tissue presented normal amounts of cortisol, THE and THF, but increased amounts of cortisone (13 and 15%) and of DHE (13%).

Rat liver tissue was investigated with corticosterone as substrate over incubation periods from 30 minutes up to six hours. Characteristic time curves were obtained for the disappearance of corticosterone and the formation of 11-dehydrocorticosterone and of ring-A reduced metabolites. Addition of thyroxin to the medium resulted in higher levels of ring-A reduced metabolites throughout the six hours of incubation. The level of 11-dehydrocorticosterone was not influenced during the first two hours but was increased at three and six hours. The effect of estradiol was similar. Tissue of rats whose liver had been damaged by gastric administration of CCl_4 or by a choline-deficient diet was also studied.

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13. INCORPORATION OF ACETATE-2-C^{14} INTO SERUM CHOLESTEROL OF PATIENTS WITH ARTERIOSCLEROSIS

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A study was made of the incorporation of acetate-2-C^{14} into the acetone-soluble lipids of blood sera, or cholesterol, in five patients with clinical arteriosclerosis, and a comparative study was made of normal controls. Satisfactory reproducibility and accuracy of the method of lipid separation was established by silicic acid chromatography. The specific activity of the free and of the esterified cholesterol fractions in sera was expressed as a function of time after the oral drink. The peak values for the free fractions were reached within one hour. The specific activity of the ester fractions gradually approached that of the free over a period of 48 hours; both then decreased at similar rates, as in the studies on dogs by Gould and his associates. Hepatic synthesis of cholesterol was calculated according to studies by Hellman on patients with malignant diseases and by Gould on those with various pathological states. Our values for patients with arteriosclerosis agreed with their values. It would therefore appear that our patients showed no alteration in cholesterol metabolism due to their disease.

14. PHOTOSCANS OF THE HEART WITH RADIOIODINATED FATTY ACID (RIFA)

John R. Evans, Ramsay W. Gunton,* Donald S. Beanlands and Robert G. Baker, Departments of Medicine and Medical Biophysics, University of Toronto, The Cardiovascular Unit of the Toronto General Hospital, and the Ontario Cancer Institute, Toronto, Ont.*

The avid extraction of fatty acid from the coronary circulation by heart muscle may be detected in photoscans of the thorax following an intravenous injection of radioiodinated fatty acid bound to human serum albumin. Photoscans of the thorax of anesthetized dogs one to four hours after injection showed selective concentration of radioactivity in the region of the heart and stomach. At necropsy, similar photoscans were obtained with the heart washed free of blood, and extracts of heart muscle showed that 95% of the radioactivity present was bound to lipid. In contrast, radioactivity in stomach was attributable to the gastric secretion of water-soluble radioiodide. Measurements of blood radioactivity after RIFA injection demonstrated rapid removal of lipid-bound radioactivity with subsequent slow clearance of recycled water-soluble radioiodide. The most favourable ratio of concentration of radioactivity in heart muscle to blood was noted about one hour after RIFA injection in well-fed dogs that received glucose and insulin at the time of injection.

Photoscans were carried out on 12 dogs two hours to six days after surgical ligation of a branch of the left coronary artery. An area of decreased concentration of radioactivity within the cardiac density pattern was noted in nine animals, but no defect was detected in the remaining three. In all cases at necropsy less radioactivity was present in the ischemic area; the ratio of lipid-bound radioactivity in samples of normal left ventricular muscle ranged from four to 13 times greater than that present in ischemic muscle. It appeared, therefore, that myocardial muscle deprived of its blood supply did not concentrate RIFA and, if the ischemic area was large enough, a region of decreased radioactivity was recognized on photoscans of the heart. Preliminary observations on human subjects have demonstrated similar results.

15. IODIDE-I^{131} BINDING BY THYROID HOMOGENATES

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Iodide-I^{131} binding by thyroid homogenates was measured by equilibrium dialysis. Cell-free calf thyroid homogenates were found to actively bind iodide-I^{131} following predialysis of the preparations. Undialyzed homogenates behaved as though they contained diffusible inhibitors and were almost completely inactive.

The total bound I^{131} present in the homogenates after equilibrium dialysis was found to be of two types. Seventy per cent was identified as I^{131}-iodinated protein, which on pancreatin hydrolysis yielded monoiodotyrosine-I^{131} and diiodotyrosine-I^{131}. The second type of bound iodide-I^{131} was found to be held by a relatively weak type of linkage; it could be readily removed from the thyroid protein by dialysis.

The effect of three thyroid inhibitors on iodide binding was studied. Perchlorate and thiocyanate, which block thyroidal iodide-concentration, completely prevented both types of I^{131}-binding by predialyzed homogenates. Thiourea, which inhibits organification of iodine by the thyroid but which does not block iodide-concentration, inhibited only iodination of protein; it did not prevent the second reversible type of iodide binding.

Cupric ion markedly enhanced iodide binding by undialyzed homogenates, but such binding was not influenced by boiling nor to any extent by perchlorate or thiocyanate. Furthermore, copper did not stimulate iodide binding by predialyzed preparations. These results suggest a non-enzymatic oxidation of iodide by copper supplemented systems.

Since cell-free thyroid homogenates bind iodide in a form that is diffusible and that is inhibited by perchlorate or thiocyanate but not thiourea, cellular integrity may not be essential for thyroidal iodide concentration.

16. THE DEVELOPMENT OF AN ARTIFICIAL PLACENTA: STUDIES ON UNBORN LAMBS TRANSFERRED TO THE ARTIFICIAL PLACENTA FOR PERIODS UP TO ONE HOUR WITH ATTEMPT AT DELIVERY TO THE ATMOSPHERE

L. Fisk, R. Hallgren, B. Boracchia, E. Maynes and J. C. Callaghan, Department of Surgery, University of Alberta, Edmonton, Alta.*

In an attempt to create an experimental preparation in which an unborn fetus was removed by Cesarean section from the mother and supported in an artificial medium, the lamb was chosen as the experimental animal. The first portion of the study consisted of transference of 19 lambs to a fluid-filled plastic chamber after first cannulating the umbilical arteries and veins with rapid take-over for an extracorporeal circulation including an oxygenator. In these animals the longest survival was 19 hours, with six of the animals surviving longer than 10 hours.

In these animals certain difficulties were present. Gradual blood destruction at the end of the sixth hour was evident, and the system required complete replacement with new donor blood. This was felt to be due to the type of blood oxygenation that was being used in this preparation. There was utilization of sugar, but enough donor glucose was provided to maintain the blood sugars at relatively normal levels.

Oxygen saturation and carbon dioxide content were studied in these animals over the period of perfusion and were found to remain within normal limits for the unborn lamb fetus.

However, attempts to deliver these animals in all instances proved unsuccessful, and it is felt that a specific study will be required with the detailed measurement of various parameters to determine the reason why these animals entered an irreversible state after a long period of perfusion.

A shorter period was chosen in the placenta, that of an hour's duration, and a more careful study was made of pO_2, CO_2 content, the electrocardiogram, the electroencephalogram, blood urea nitrogen, and pH at 10, 20, 40 and 60 minutes of perfusion. At the termination of this period an attempt was made to deliver these animals to the atmosphere to try to obtain a long-term survivor. There were 10 animals in this group. One of these animals became a long-term survivor, and the details of the perfusion of this animal and the many connected studies are presented and a comparison is undertaken between the long-term survivor study and the study of the other nine animals who, for one reason or another, failed to become complete survivors.

17. THE EFFECT OF POSTURE ON HEMOGLOBIN, HEMATOCRIT AND PLASMA PROTEINS

T. Greidanus and J. A. L. Gilbert, Department of Medicine, University of Alberta, Edmonton, Alta.*

The daily fluctuations in hemoglobin value are usually attributed to variations in the technique of estimation. Where day-to-day changes in the hemoglobin are used as a measure of the patient's progress, these variations may be of profound importance.

Assumption of the upright posture was shown, as early as 1928, to be associated with a fall in plasma volume. That such changes in plasma volume might be associated with alterations in the hemoglobin, hematocrit and plasma proteins is the basis of the present study.

Sixty-seven subjects were studied. This group was made up of a control group of 24 patients and 43 patients hospitalized for various reasons in the University of Alberta Hospital. A further four subjects remained horizontal throughout the test and served as a control group.

Hematocrit and hemoglobin estimations were made at bedrest, then at 15, 30, 60, 90 and 120 minutes after assuming the upright posture. Hemoglobin and hematocrit determinations were made in triplicate. The increase in hemoglobin varied between 2.6 and 14.3%, averaging 8%, while the increase in hematocrit was between 2.5 and 25.5%, averaging 8.2%. This increase was appreciably greater in underweight subjects than in those who were overweight. The effects of food, adrenaline and return to the recumbent position were also studied.

The following conclusions were reached. Assumption of the upright position is associated with a significant rise in hemoglobin, hematocrit and plasma proteins. These changes are more marked in underweight people. The consumption of food and water causes slight changes in hemoglobin and hematocrit. In the daily hemoglobin estimations the most reliable results will be obtained when blood samples are drawn before the patient gets out of bed.

18. PRE-PULMONARY BYPASS: FURTHER STUDIES OF VENOVENOUS PERFUSION WITH THE TRACHEOBRONCHIAL TREE FILLED WITH NITROGEN AND SALINE

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In order to study the effectiveness of venovenous perfusion in complete support of respiratory function, normal gas exchange was prevented by filling the tracheobronchial tree with either nitrogen or normal saline.

Mongrel dogs, weighing 6 to 11 kg., were anesthetized by pentobarbital administration and were heparinized. They were then subjected to catheterization of both proximal and distal ends of both external jugular and femoral veins. The returning venous blood was oxygenated in a rotating disc oxygenator and delivered by gravity into the right atrium. The tracheobronchial trees were filled with nitrogen in 10 dogs and with normal saline in 10 dogs. With respiratory function thus obstructed, the dogs were perfused for a period of 60 minutes. During this period pO_2, CO_2 content, pH, respiratory rate, electrocardiographic tracing, electroencephalographic tracing and arterial pressure were monitored.

In Group A (nitrogen), eight of the 10 dogs were long-term survivals. Flow rates averaged 38.9 ml. per kg. per minute. The peripheral oxygen tension varied between 28.8 and 44.8 mm. Hg, the CO_2 content between 32.0 and 39.5 vol. %. The pH varied from 7.20 to 7.5.

In Group B with normal saline in their lungs, four of the 10 dogs were long-term surivors. Flow rates averaged 38.6 ml. per kg. per minute. The peripheral arterial pO_2 varied from 28.3 to 43.7 mm. Hg, the pH between 7.52 and 7.0, and the CO_2 content between 33.6 and 38.9 vol. %.

Although the number of dogs studied is relatively small, a deleterious effect appeared to occur as the result of filling the tracheobroncheal tree with liquid (saline) as compared to an inert gas (nitrogen) under conditions of support by prepulmonary bypass.

19. A DEFECT IN THROMBOPLASTIN GENERATION ASSOCIATED WITH THE MENSTRUAL CYCLE

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Serum prepared from normal females at the time of the menses is defective when tested in the thromboplastin generation test. This abnormality appears four to five days before the onset of menstruation and disappears at its completion. Postmenopausal females do not have this cyclic defect. During pregnancy the abnormality is not evident but near term it reappears. Some males, in particular those who have repeated gastrointestinal or genitourinary hemorrhage, demonstrate a similar serum change, although non-cyclic. It is most pronounced when phospholipid is used instead of platelets as the lipid source in the thromboplastin generation test. In some cases normal generation can be achieved when adequate platelet concentrations are used. Platelet fractionation studies have as yet failed to demonstrate the platelet factors that modify this defect. The abnormal generation is not due to lack of a known clotting factor, as correction is achieved by addition of normal serum or serum deficient in factor IX, factor X and factor XI. Absorption with asbestos fibres improves this serum activity, in contrast to the behaviour of factor IX and PTA deficient sera. Minute concentrations of thrombin that will not clot plasma in six minutes will, when added to the serum, correct the defect. The correcting effect is lost if the serum and thrombin are incubated together before testing. Increased prothrombin consumption can be demonstrated during the periods when the serum is abnormal. Changes in prothrombin and antithrombin balance would appear to be important. Cyclic hormonal changes are certainly related, but preliminary attempts to reproduce the defect by administration of hormones have been inconclusive.

20. ACTH RESPONSIVENESS AND SIGNIFICANCE OF PLASMA CORTISONE IN THE HUMAN NEWBORN

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The level of individual corticosteroids in the cord plasma of the human newborn may reflect either the secretion of the neonatal adrenal, the placental transfer of maternal hormones, a combination of both, or the product of some of their metabolic transformations by the placenta as well as by the newborn itself. During the first few days of life the capacity of the newborn adrenal to respond to stress is probably conditioned by such factors as the rapid morphological changes of the fetal cortex and the progressive withdrawal of maternal steroids present at birth. Previous studies of the functional capacity of the neonatal adrenal have led to conflicting interpretations, owing in part to the lack of specificity of the steroid assays available at the time.

In the present study plasma cortisol, cortisone and corticosterone were measured by double isotope derivative assay in maternal, cord and neonatal plasma. The steroids were purified to constant isotope ratio (H^3/C^{14}) by sequential paper chromatography in four different systems. The appropriate 4-C^{14} steroid provided internal correction for losses from the time of extraction. Quantitative results were obtained by measurement of the tritiated 21-acetoxy, steroid derivative.

The following results were obtained:

1. The cortisone concentration in cord plasma is approximately three times greater than in maternal plasma.

2. Cortisone remains quantitatively the major corticosteroid in newborn plasma during the first week of life. (Studies were not prolonged beyond this point.)

3. The mean hydrocortisone concentration in cord plasma is seven times less than that of maternal plasma.

4. ACTH administration during the first three days of life produces a marked rise in plasma hydrocortisone and a smaller, but significant, rise in plasma cortisone.

5. Corticosterone, while detected in maternal plasma, is only measurable in newborn plasma following ACTH stimulation.

21. URINARY RING D-α-KETOLS IN THE HUMAN SUBJECT

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Little or nothing regarding the level of ring D-α-ketolic estrogens (16α-hydroxyestrone, 16β-hydroxyestrone and 16-ketoestradiol-17β) in the urine of non-pregnant humans has been reported to date. Methods involving column partition chromatography and fluorimetric analysis were employed for the measurement of these compounds. It was found that in nine normal males (17-50 years) the ring D-α-ketols measured 2.3-10.1 μg./24 hr. (mean = 5.6 μg./24 hr.). In 12 normal postmenopausal women (47-72 years) the corresponding values were 1.3-4.2 μg./24 hr. (mean = 2.8 μg./24 hr.). Further investigation of a pooled fraction indicated that 16α-hydroxyestrone accounted for some 50% of the ring D-α-ketols, the remainder 16-ketoestradiol-17β and/or 16β-hydroxyestrone. In order to investigate the formation of ring D-α-ketols *in vivo*, estrone-16-C^{14} was injected into three subjects. The ring D-α-ketolic fraction from the partition column was reduced chemically with sodium borohydride, yielding 16-*epi*estriol (from 16-ketoestradiol and/or 16β-hydroxyestrone) and estriol (from 16α-hydroxyestrone). The specific activities of these two fractions were equal throughout the experimental period. This suggested that these ring D-α-ketols were arising from estrone, either directly or via some common intermediate. Additional evidence in favour of this view was obtained by injecting 16-ketoestradiol-17β-16-C^{14} and 16-*epi*estriol-16-C^{14} into each of two subjects. In neither case was significant activity present in the urinary 16α-hydroxyestrone. This indicated negligible production of the latter from 16-ketoestradiol-17β either directly or via the triols.

22. MALVARIA

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Irvine in 1961 extracted certain substances from the urine of schizophrenic patients. Although these have not yet been identified, they are readily demonstrated by Irvine's technique or by the method of Hoffer and Mahon (1961). These unidentified substances, "US", have not yet been found in any subject who is well. "US" was found in the following proportion in the conditions listed:

Acute schizophrenia	75%	(N >100)
Chronic schizophrenia	50%	(N >100)
Mentally retarded children	30%	(N > 40)
Other psychiatric diagnoses excluding schizophrenia	16%	(N >100)
Normal and surgically stressed patients	2%	(N > 50)

In order to produce a homogeneous diagnostic group Hoffer and Osmond (1962) have described the state of subjects who have "US" in their urine as malvaria. This is a chemically based diagnosis. Malvariacs, no matter what their psychiatric diagnosis, are homogeneous with respect to a psychological test developed by Hoffer and Osmond (1961) and with respect to clinical findings. When malvariacs recover, the urine factor disappears; when they relapse, it reappears.

Out of a group of over 100 malvariacs 75% were diagnosed as having schizophrenia by the usual clinical criteria. The remainder included diagnoses of depression, anxiety state, alcoholism, psychopathic personality and mental retardation.

All of the malvariacs that the author has so far treated (N >100) have responded to the same form of treatment found to be effective for schizophrenics. The chemical assay was helpful in guiding treatment. The diagnosis of malvaria seems to be a more useful one than that of schizophrenia, since it is free of subjective bias.

23. CHOLESTEROL BIOSYNTHESIS IN SKIN: THE EFFECTS OF A CHOLESTEROL INHIBITOR, TRIPARANOL

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The synthesis of cholesterol from acetate has been carefully studied, and the pathway, as far as the cyclization of squalene and formation of lanosterol, is generally agreed upon. Of the steps from lanosterol to cholesterol, however, there are many gaps in our knowledge. The advent of triparanol, initially introduced to lower blood cholesterol levels, has resulted in some important theoretical contributions in understanding the last steps in the biosynthesis of cholesterol.

By means of chromatographic techniques, normal rat skin was shown to contain a number of intermediates of cholesterol synthesis, namely, lanosterol, dihydrolanosterol, methostenol, dehydromethostenol, △7 cholestenol, 7 dehydrocholesterol, desmosterol and cholesterol. In each instance there was a preponderance of the C24-25 saturated compounds. After ingestion of triparanol, there was a sharp decrease in the C24-25 saturated compounds and an increase in the C24-25 unsaturated members. We also noted the appearance of a new compound believed to be △7, △24 cholestenol. Radio-

activity tended also to concentrate in the compounds with an unsaturated C24-25 bond.

Our data indicate that sterol intermediates with an unsaturated C24-25 bond are found in untreated rat skin, and that saturation of this bond may normally be a final step in the biosynthesis of cholesterol. Triparanol inhibits a reductase enzyme (or group of enzymes) which may act at the C24-25 position in several closely related sterols of differing nuclear configuration.

24. EXPERIENCE WITH A METHOD FOR THE STUDY OF CALCIUM ABSORPTION FROM THE HUMAN GUT USING A STANDARD DOSE OF CALCIUM LABELLED WITH CALCIUM-47

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In an attempt to find a convenient method for study of calcium (Ca) absorption from the gut in various clinical and experimental conditions, a standard test making use of Ca^{47} as a tracer has been devised. Three hundred milligrams of stable Ca (as lactate) in aqueous solution mixed with 35 microcuries (μc.) of Ca^{47} is given orally to a fasting subject kept on normal diet containing 700-1200 mg. of Ca per day. No meal is taken for 2½ hours after ingestion of the dose. Stable and radioactive Ca were measured for six days in plasma, urines and stools. Thirteen tests were performed on nine normal adults between 23 and 41 years of age and 25 tests on 12 patients with various disturbances of Ca metabolism. Two parameters were evaluated: % radioactivity absorbed from the gut (equal to dose administered minus % dose excreted in stools in six days) and plasma radioactivity peak, usually reached within two hours after dose ingestion, expressed as % radioactivity dose per litre of plasma.

In normal adults, absorption ranged from 31.9 to 43.6% and the plasma peak from 1.22 to 2.30% dose per litre. Repeated twice in one subject and three times in another when on various Ca intakes, the test gave results falling within the above ranges. In five patients with malabsorption (three with symptomatic osteomalacia) absorption prior to treatment was variably reduced (8.8-31.6%) but the plasma peak was consistently low (0.36-0.69 % dose per litre). A gluten-free diet with or without vitamin D resulted in a marked rise in % absorption (up to 72.9%), but until the osteomalacia healed, the plasma peak remained low. In three patients with osteoporosis (all over 50 years of age) absorption (25.6-30.7%) and plasma peaks were slightly below normal. In two patients treated with methandrostenolone (Danabol) absorption increased from 30.7 to 67.8% and from 25.6 to 39.0%. Tests were done on patients with uremia, idiopathic nephrocalcinosis, a patient with gastric carcinoma before and after gastrectomy, and a normal adult when on very low and very high Ca intakes. So far results have correlated well with clinical conditions.

The value of the method described may consist in combining study of intestinal Ca absorption with that of endogenous Ca metabolism, as the latter appears to influence the fate of the absorbed portion of Ca^{47} and the behaviour of Ca^{47} plasma peak. Thus, interdependence of absorption and metabolism of Ca may be conveniently studied.

25. AN ADDITIONAL SITE OF ACTION OF THE FOLIC-ACID ANTAGONIST, METHOTREXATE (AMETHOPTERIN)

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It has been widely held, on the basis of animal experiments, that the folic-acid antagonist, methotrexate, acts by irreversibly inhibiting the enzyme dihydrofolic reductase. However, recent experiments carried out in man suggest that another important mode of action for this drug may be competition for the folic acid cell entry mechanism. Three subjects, a normal human volunteer, a patient with acute myelocytic leukemia and a patient with choriocarcinoma, were each pre-loaded with methotrexate (10 mg. per day). Fifteen micrograms (μg.) per kg. of tritium-labelled folic acid was then given intravenously to each subject and the 24-hour urinary excretion of folic acid radioactivity was determined. A mean value of 60.6% of radioactivity was excreted *vs.* a mean of 18.0% for 10 non-methotrexate-loaded control subjects. Similar results were found when the pre-loading was carried out with non-labelled folic acid rather than with methotrexate.

However, when the order of administration of the compounds was reversed, the behaviour of methotrexate and folic acid differed markedly. If 15 μg. per kg. of labelled folic acid was given initially to normal subjects and followed by a "flushing dose" of unlabelled folic acid (30 mg.) at 24 hours, a mean of 36.0% of the folic acid radioactivity was displaced and recovered in the urine, as compared with only 4.6% when the flushing agent was unlabelled methotrexate. It appears therefore that methotrexate competes strongly for the carrier system utilized by folic acid to *enter* cells and that this competition may constitute an important part of its activity. The weak displacing activity of methotrexate for *intracellular* folic acid confirms indirectly the finding of other workers that high intracellular methotrexate levels are difficult to obtain and that the transport of methotrexate into cells is slow. Methotrexate therefore can compete efficiently for the folic acid carrier system; it is unable, however, to utilize this system efficiently in order to penetrate the cells.

26. SUSCEPTIBILITY OF DIABETIC AND NON-DIABETIC RATS TO ATHEROGENIC DIET

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It is a clinical observation that the incidence of atherosclerosis is higher in the diabetic than in the non-diabetic population. It is not clear, however, whether this is the result of the recognized biochemical abnormalities associated with a relative insulin deficiency or an integral part of a larger "diabetic syndrome". The incidence of atheromatous lesions produced by a high-fat diet has been studied in alloxan diabetes, a condition due specifically to insulin deficiency. Groups of animals were sacrificed at intervals during administration of an atherogenic diet. Measurements were made of plasma proteins, cholesterol, total phospholipids and phosphatidyl ethanolamine and of certain coagulation factors (factors V, VII, VIII, X, antithrombin) and coagulation times (prothrombin, recalcification, Stypven). Histological examinations of heart and coronary arteries were made. It was found that diabetic animals had a food intake almost identical to that of non-diabetic animals. Despite this, the plasma concentrations of cholesterol, total phospholipids, and phosphatidyl ethanolamine were consistently higher in diabetics than in non-diabetics. The concentrations of the coagulation factors were increased equally in diabetics and non-diabetics. Atheromatous lesions of coronary arteries were more frequent, more extensive and occurred after a shorter period of the experimental diet, in the diabetics. It is concluded that: (1) insulin deficiency leads to increased susceptibility to atheromatous lesions; (2) the increased susceptibility is related to the plasma level of lipids rather than to a hypercoagulable state; and (3) the hypercoagulable state is not related to the plasma concentrations of total phospholipids or phosphatidylethanolamine.

27. CALCIUM METABOLISM IN RENAL FAILURE

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The present study attempts to delineate the changes in calcium metabolism in patients with severe chronic renal disease. These changes were evaluated in the following ways: (1) Standard absorption was measured, using a tracer dose of calcium-47 in controls and patients with mild and severe renal failure. Unabsorbed calcium was measured by fecal counting over four days, and blood activity curves were also obtained. (2) Distribution, kinetics and excretion in stool and urine were determined in these same groups after intravenous administration of calcium-47. (3) Analysis was made of the fourth lumbar vertebra from control subjects without renal disease, patients dying of acute renal failure and patients dying from chronic renal failure. Mineral and nitrogen analyses were expressed as per unit volume of original bone. (4) Careful dietary histories were taken on the patients studied to determine their individual calcium intakes at home on unrestricted diets.

Information from these different aspects was integrated into a dynamic picture of changing calcium metabolism in these patients as their disease progressed. Evidence of impaired calcium absorption as renal failure worsens and, at the same time, over-mineralization of vertebral bone were found by analysis. Contrary to what might have been expected, bone formation rate was not increased, which suggested that an abnormality in bone resorption is present in these patients.

28. THE EFFECT OF DEXAMETHASONE AND NITROGEN MUSTARD ON THE PRODUCTION OF RHEUMATOID FACTOR IN RHEUMATOID ARTHRITIS

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Dexamethasone, administered in large doses to six patients with rheumatoid arthritis, was found to reduce the amount of circulating rheumatoid factor, as measured by the sheep cell agglutination and latex agglutination reactions.

Nitrogen mustard, administered in the usual therapeutic dose to 12 patients with rheumatoid arthritis, was also found to reduce the amount of circulating rheumatoid factor, but to a less marked and consistent degree.

In each instance, the fall in sheep cell agglutination and latex agglutination titres did not occur for 10 days and was most pronounced in about 30 days.

It is suggested that both adrenocorticosteroids and nitrogen mustard have the capacity to suppress the formation of rheumatoid factor in rheumatoid arthritis.

29. STUDIES ON THE *IN VITRO* ACTIVITY OF THYROID-STIMULATING HORMONE (TSH)

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Bottari has proposed that the *in vitro* accumulation of radioiodine and its subsequent release under the influence of thyroid-stimulating hormone (TSH) can be used as an assay method for TSH levels in human serum. The periods of incubation used

for his studies would seem to introduce a probable source of error despite the fact that tissue survival can be demonstrated for these periods. Studies were carried out using the *in vivo* accumulation of radioiodine and attempts at its *in vitro* release with TSH. This modification was felt to help overcome the hazards of tissue change with prolonged incubation periods.

The results obtained using this method as well as Bottari's original method show that radioiodine accumulates in the medium independent of the presence of TSH. The addition of TSH to the system did not appear to increase the rate of spontaneous radioiodine discharge or diffusion. With the particular experimental model under study TSH did not appear to activate the hydrolysis of thyroglobulin. The value of this type of approach in the assay of TSH is questioned. The form which the spontaneously discharged radioiodine is in is under study with chromatographic techniques.

30. SEGMENTAL RESPONSES OF THE GASTROESOPHAGEAL SPHINCTER TO INCREASES IN INTRAGASTRIC PRESSURE

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In studies on 18 healthy human subjects, intraluminal pressures were recorded by means of four water-filled polyethylene tubes, each having a lateral orifice. A fifth tube was used for aspiration of the esophagus. Respiratory movements were recorded by means of a tubular pneumograph placed about the subject's chest. The pressure-detecting units and the pneumograph were connected to strain gauges, the outputs of which were led to a suitable recorder.

The gastroesophageal junctional zone was first defined by a continuous recording of resting pressures while the units were withdrawn from the stomach into the esophagus. A dilute solution of methylene blue was infused into the stomach and a pneumatic cuff placed about the abdomen. The units were positioned such that pressures were recorded from the esophagus, the suprahiatal and the infrahiatal portions of the sphincter and the stomach. A series of recordings were made while pressure was applied to the abdominal wall. The esophagus was aspirated after each increment in abdominal pressure.

A zone of elevated pressure was found interposed between the stomach and esophagus. The characteristics of this zone were similar to those reported by other investigators. As the abdominal pressure was increased, there was a simultaneous elevation in pressure in the stomach and both portions of the sphincter. The pressure in the esophagus remained unchanged. Reflux occurred on one occasion in three subjects. In two instances reflux was preceded by a swallow.

31. A STUDY OF BLOOD VOLUME OF THE NEWBORN INFANT DURING THE INITIAL NEONATAL PERIOD

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The purpose of this study was to define the blood volume characteristics of the normal infant at delivery and at 24 hours, in order to demonstrate the changes occurring during the initial neonatal period. One hundred and eleven normal mature infants were studied, 50 at delivery and 61 at 24 hours after delivery.

Plasma volume was measured by the Evans blue dye dilution technique, and blood volume was calculated with the total body hematocrit.

The mean levels for the intravascular volumes at birth were: plasma volume 41.5 c.c., cell volume 35.0 c.c. and blood volume 76.5 c.c. per kg. of body weight. There was a 10% increase of plasma volume to 45.6 c.c.; a 7.5% increase of cell volume to 37.7 c.c.; and a 9% increase of blood volume to 83.3 c.c. per kg. of body weight during the initial 24 hours of the neonatal period.

The significance of these blood volume changes to the individual infant was assessed by means of blood volume estimations performed at birth and repeated at 24 hours in 25 normal infants.

Three patterns of adjustment were observed. The plasma, cell and blood volumes remained unchanged in 50% of cases. The second group, representing 25%, demonstrated a significant rise of plasma volume of 6.1 c.c. per kg. of body weight. The third group, representing 25% of cases, demonstrated a moderate rise of plasma volume of 15.5 c.c., a small but significant rise of cell volume of 7.0 c.c., with a resultant increase of blood volume to 22.6 c.c. per kg. of body weight.

32. A LOW-TEMPERATURE TISSUE HOMOGENIZATION TECHNIQUE

B. Ludvigsen and G. Monckton (introduced by D. R. Wilson), Neuro-Muscular Research Unit, University of Alberta Hospital, Edmonton, Alta.

A low-temperature tissue homogenizer that produces total cellular disruption with liberation of cell contents was used in this study. This technique provided a frozen block of cell-free cell contents without the disadvantage of added diluent. The homogenates of muscle so obtained could be stored under deep-freeze conditions without significant deterioration of enzyme content, with the exception of LDH.

Selected muscle enzyme assays were carried out on muscle homogenates from normal and dystrophic subjects and from relatives of dystrophy patients. The normal ranges of aldolase (ALD), phosphohexose isomerase (PHI), glutamic oxalacetic

and pyruvic transaminases (GOT, GPT), and lactic dehydrogenase (LDH) were compared with the levels seen in the described case material and also with the serum levels of the same enzymes.

The results suggested reduced intracellular ALD, PHI, and GOT in some patients with muscular dystrophy, but no distinctive pattern for the other enzymes assayed, some patients having normal and some reduced levels irrespective of those for ALD, PHI and GOT.

33. IMPAIRMENT OF CARDIOVASCULAR RESPONSE TO TYRAMINE BY RESERPINE ADMINISTRATION IN MAN

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Reserpine has not been shown to deplete tissue catecholamines in man, although this effect is well established in animals. Since tyramine produces its effect by releasing endogenous noradrenaline at sympathetic nerve endings, the response to tyramine has been used to demonstrate that reserpine in therapeutic doses depletes tissue noradrenaline. Resting supine blood pressure was recorded continuously in 12 patients; tyramine, 0.2 mg./kg., was given intravenously after the blood pressure had stabilized. The pressor response to tyramine was the statistic used for comparison. In eight patients, reserpine, 0.03 mg./kg., was given intravenously after the first study and again 24 hours later, and the observations were repeated 48 hours after the initial study. In five patients, no reserpine was given, and their responses on the two occasions served as additional control observations.

Cardiac output was determined, using indocyanine green, at intervals before and after tyramine on both days in 10 of the 12 patients, and the total peripheral resistance was calculated from these data.

The pressor response to tyramine was significantly less on the second occasion in the patients receiving reserpine, in contrast to the control patients, whose response did not differ significantly on the two occasions. Cardiac output changes after tyramine were variable and were not altered by reserpine. In the reserpinized patients, the increase in total peripheral resistance produced by tyramine was significantly less on the second occasion.

Thus, reserpine in therapeutic doses, was demonstrated to impair cardiovascular responses which are known to depend on intact noradrenaline stores.

34. HUMAN GROWTH HORMONE — LIKE EFFECT OF OVINE PROLACTIN IN MAN

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Growth hormone preparations from other than primate sources are biologically inactive in man. Recent chemical, biological and immunological evidence suggests that human growth hormone is identical or similar to human prolactin. The metabolic effect of a purified sheep prolactin preparation was therefore studied in man. Three hypopituitary dwarfs on metabolic balance study were given human growth hormone and subsequently sheep prolactin. The sheep prolactin preparation was found to mimic the metabolic action of human growth hormone, producing nitrogen retention, hypercalciuria and relative impairment of carbohydrate tolerance. One 18-year-old girl with a bone age of 12 years and a prepubertal body habitus received a second course of sheep prolactin after a five-month interval, and hypercalciuria and nitrogen retention again occurred. No antibodies could be demonstrated in the patient's serum before, during or after the second course of prolactin. After an additional interval treatment with prolactin was again instituted and has been continued to the present time. While hemagglutinating antibodies in low titre appeared after one month of the third course of prolactin, the patient showed no clinical evidence of resistance or hypersensitivity. She has grown a quarter of an inch and has now very definite evidence of the development of mammary glandular tissue. Sheep prolactin also produced hyperglycemia and nitrogen retention in a hypophysectomized juvenile diabetic, a change previously reported to occur also with human growth hormone.

35. GEL FILTRATION OF THYROTROPIN AND THE LONG-ACTING THYROID STIMULATOR OF GRAVES' DISEASE

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A "long-acting thyroid stimulator" may be assayed in the blood in Graves' disease, and its relationship to thyrotropin has been the subject of recent investigations. Further data on this problem were obtained with the use of the technique of gel filtration on Sephadex G-200.

Hypophysial thyrotropin (bovine, Morris, or human, Condliffe) had a distribution coefficient (K_d) in Sephadex G-200 slightly greater than albumin, i.e. about 0.75. When human thyrotropin was added to normal human serum and filtered, the usual three protein fractions were obtained; thyro-

tropic activity was with the third fraction, which was predominantly albumin. Fractionation of serum containing the long-acting thyroid stimulator, however, showed that activity to be mainly with the second fraction (K_d about 0.45), the major component of which, by starch gel and immunoelectrophoresis, was gamma globulin.

One theory explaining the nature of the long-acting thyroid stimulator is that it is thyrotropin bound in an abnormal way to plasma protein or proteins. The different K_d of these substances on Sephadex G-200 facilitated the attempt to break this hypothetical bond by means of concentrated (1 or 2M) sodium chloride or 6M urea solutions. Equilibrium and filtration of plasma in these solvents, however, failed to affect either the K_d or the prolonged action of the contained thyroid stimulator. Thus no data were obtained identifying thyrotropin and the long-acting thyroid stimulator, but further points of dissimilarity were disclosed.

36. UNEXPECTED CLINICAL ASSOCIATIONS WITH POLIOVIRUS INFECTION

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Poliovirus III was recovered from feces and rising homotypic antibody titres were demonstrated in an infant who presented with drowsiness, left-sided seizures followed by spasticity which improved slowly over several weeks, and pleocytosis of the cerebrospinal fluid. Poliovirus I has been recovered from two children who presented with paraplegia, both of whom received at least three Salk vaccinations, and Poliovirus II was recovered from an unvaccinated infant who developed paraplegia. Between 1958 and 1962, we studied eight patients with paralysis due to Poliovirus I following three or four doses of Salk vaccine.

During 1962, seven patients developed febrile upsets within 30 days after oral administration of trivalent Sabin attenuated poliovirus vaccine. Although three patients showed lymphocytosis of cerebrospinal fluid, and two had muscular weakness, no paralysis was encountered. Feces of two patients yielded Poliovirus I, another four patients excreted Poliovirus III and one patient excreted all three serotypes simultaneously. All viruses showed aluminum marker characteristics identical with attenuated vaccine viruses, except one Type I virus which was however inactivated following incubation at 40° C., in common with attenuated strains. Three unvaccinated patients who contracted paralytic poliomyelitis during 1962 excreted Poliovirus Types I, II and III, respectively, all of which showed aluminum markers typical of virulent strains.

37. CITRULLINURIA: A NEW METABOLIC DISORDER ASSOCIATED WITH MENTAL DEFICIENCY

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An abnormal aminoacid present in large quantities, up to 3 g. per day, has been isolated and identified as L-citrulline. On the basis of high concentrations of citrulline found in the body fluids of the patient, the defect appears to be of metabolic origin. It has been suggested that the condition arises from a metabolic block in the utilization of citrulline, although the finding of normal concentrations of urea in the patient's blood and urine makes it appear unlikely that the conversion of citrulline into arginino-succinic acid by the liver is the site of the lesion.

When the patient's protein intake was increased or decreased, the urinary extraction of urea changed in the same direction. There was a tendency for the blood levels and the urinary excretion of citrulline to follow this same pattern, and this probably explains the previously reported variability in the daily excretion of citrulline. The concentration of citrulline in the blood remained relatively constant. An oral load of citrulline caused an increase in the concentration of citrulline in both blood and urine. Clinically, the child presented as a case of severe mental retardation with a tendency to episodes of vomiting when his protein intake was high.

38. THE USEFULNESS OF SERIAL PLASMA CORTICOID DETERMINATIONS

Beverley P. Murphy and Chauncey J. Pattee, Clinical Investigation Unit, Queen Mary Veterans' Hospital, Montreal, P.Q.*

The determination of large numbers of plasma samples for the assessment of adrenocortical function was made feasible by the introduction of a simple, highly specific method for the measurement of plasma-free corticoids (cortisol and corticosterone) requiring only 1 ml. of plasma or serum per sample (B. P. Murphy, W. Engelberg and C. J. Pattee—in press). With a slight modification, it may also be used to determine 11-desoxycortisol. The application of this method was investigated with particular reference to studies of diurnal variation, ACTH stimulation and suppression, and response to mepyrapone (Metopirone).

Studies of diurnal variation gave 9:00 a.m. values of 8-24 μg. corticoids per 100 ml. in healthy subjects, values <8 in most cases of adrenal insufficiency and in a few cases of advanced cirrhosis, and values >30 in cases of Cushing's syndrome and in subjects receiving estrogen. Night values were much lower than morning values except in cases

of Cushing's syndrome and in moribund patients. After ACTH stimulation, values rose to 30-60 μg. per 100 ml. in healthy subjects and in those with non-endocrine disease, but rose only slightly or not at all in subjects with hypopituitarism and Addison's disease. Values >100 were observed in subjects receiving estrogen. When ACTH was suppressed by dexamethazone administration, values fell to 0-5 μg. corticoids per 100 ml. except in cases of Cushing's syndrome. When mepyrapone was administered intravenously for four hours, plasma cortisol fell rapidly to 0-5 μg. per 100 ml. while plasma desoxycortisol rose to a peak of 8-15 μg. per 100 ml. by six hours in healthy subjects but failed to rise in those with hypopituitarism. In these procedures, changes in plasma levels could be followed as often as half-hourly. The results in all cases correlated well with other clinical and laboratory data.

39. THE MEASUREMENT OF THE BINDING CAPACITY OF CORTICOSTEROID-BINDING GLOBULIN (CBG) IN HUMAN PLASMA

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A method was developed for the determination of corticosteroid-binding globulin (CBG) in terms of the amount of cortisol required to saturate it at 9° C. and at 37.5° C. At low concentrations of cortisol it was shown that there was no unbound cortisol present in plasma. After a certain amount of cortisol was added, the % unbound cortisol increased with further additions of steroid in proportion to the amount of binding protein present. A linear relationship between the % unbound and the log total cortisol was demonstrated. Extrapolation of the straight line so obtained back to zero % unbound gave the amount of cortisol required to "saturate" the protein under the conditions used. Determinations of CBG binding capacity made at 37.5° C. were technically much more difficult to perform and, since they were consistently 9% of those made at 9° C., the measurement at 9° C. was adopted for routine use.

Values of CBG binding capacity at 9° C. in human plasma gave a mean of 31.8 ± 5.5 μg. cortisol/cap./per 100 ml. in healthy men, 30.9 ± 4.5 in healthy women, and 24.3 ± 5.1 in male subjects with Laennec's cirrhosis. With estrogen administration, values rose to approximately twice the previous levels in all groups. No change was found after the administration of ACTH. Unlike plasma corticoid levels, CBG levels did not undergo a diurnal variation. A good correlation was found between CBG binding capacity and plasma corticoid levels (cortisol and corticosterone) when the latter were determined at the same time of day. CBG values were found to be clinically useful in the assessment of adrenal and hepatic disease.

40. THE EFFECT OF ADRENALINE ON BLOOD COAGULATION, PLATELET ECONOMY AND THROMBUS FORMATION

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In the present study, the effects of adrenaline on clotting, platelet economy and thrombus formation were examined. The doses of adrenaline used ranged from 1-150 μg. per kg. body weight. The intravenous injection of large doses of adrenaline in pigs, rabbits and dogs did not shorten the clotting time in glass or silicone-coated tubes or accelerate thromboplastin formation, while the administration of smaller doses did. These changes could be reproduced *in vitro* by the addition of buffered adrenaline solution to blood.

The effect of daily injection of adrenaline on blood coagulation and platelet economy in the rabbit was studied by means of the clotting time, factor VIII, factor IX, factor XII assays and the DFP^{32} technique for platelet survival. Moderate doses of adrenaline over a 14-day period increased clotting activity, shortened platelet survival by about 50% and increased platelet turnover twofold. Larger doses of adrenaline did not cause any increase in clotting activity or change in platelet survival, but platelet turnover was increased.

The relationship of these findings to thrombus formation in a high flow pulsatile system was studied, using a quantitative extracorporeal shunt technique. These studies, which were carried out in pigs and rabbits, showed that the intravenous administration of adrenaline in doses of one to 30 μg./kg. body weight increased thrombus formation twentyfold. Larger doses of adrenaline did not cause any increase, and with doses greater than 100 μg./kg. body weight there was little change. Doses of adrenaline that accelerate clotting, shorten platelet survival and enhance the formation of thrombi. This effect seems to be mediated through an effect on the blood and platelets rather than on the vessel wall.

41. MEASUREMENT OF CORONARY BLOOD FLOW USING 4-AMINO ANTIPYRINE

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Extensive application of the N_2O method of measuring coronary flow is limited both by the

length of the period over which observations must be continued and by the time-consuming nature of the analytical techniques. For this reason a simpler technique was developed by modification of the method for measuring regional blood flow suggested by Huckabee. 4-Amino antipyrine (4 A-A), an inert substance which diffuses rapidly into tissue fluids, is infused intravenously at a diminishing rate to obtain an arterial-coronary sinus (A-CS) difference which is initially large but is reduced by 2½ minutes to zero. The mean A-CS concentration difference of 4 A-A is obtained by withdrawal of blood from brachial artery and coronary sinus using identical sampling systems and sampling rates for three minutes. Coronary circulation time is determined by use of platinum electrodes and sodium ascorbate, and an appropriate correction is made to the mean concentration difference. Spot samples drawn at three minutes from each site show that equilibrium has occurred. By means of myocardial biopsy the partition coefficient between heart muscle and venous blood in the dog has been shown to be approximately 1, even during rapidly changing venous concentration. Consequently, tissue concentration may be derived from coronary sinus concentration. Measurement of coronary flow thus occupies only three minutes. Analysis of samples for 4 A-A involves a simple spectrophotometric technique, and the whole procedure is considerably less time-consuming than present N_2O methods. The mean coronary flow in 13 dogs was 124 ml./100 g./min. Nitroglycerine caused no systematic change in coronary flow. By contrast dipyridamole (Persantin) repeatedly caused an increase.

42. TYROSINE METABOLISM IN PHENYLKETONURIA

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Measurements of the fasting plasma tyrosine level in phenylketonuria have shown that this is higher than normal in the baby with the untreated disease, slightly lower than normal in the older, untreated patient and lower still in the patient treated with a low phenylalanine diet (see Table). These variations are not wholly explained by variations in the dietary tyrosine intake. Other factors which may be responsible are the primary metabolic block in phenylalanine metabolism which is characteristic of phenylketonuria and the secondary block in tyrosine metabolism caused by high concentrations of phenylalanine in the blood. This latter block can be overcome either by feeding a low phenylalanine diet or by giving extra tyrosine, as demonstrated by the effect of these two treatments on pigmentation of the hair.

Some of these variations in plasma tyrosine level can be related to the urinary excretion of certain catecholamines. In older, untreated patients the excretion of adrenaline and dopamine is less than in controls (W. Kalow and M. W. Partington—unpublished data, 1961); the values return towards normal after treatment with a low phenylalanine diet (H. L. Nadler and D. Y.-Y. Hsia, *Proc. Soc. Exper. Biol. and Med.*, 107, 721, 1961). The effect of extra dietary tyrosine is under study.

FASTING PLASMA TYROSINE LEVELS* IN PHENYLKETONURIA

Subjects	Age range	Number of: Patients	Observations	Plasma tyrosine level (mg./100 ml.) Mean	Standard deviation
Normal controls	4 days-43 years	50	50	1.42	0.36
Phenylketonuria:					
Untreated	11 days-11 months	8	13	2.45†	1.54
Untreated	1-54 years	63	63	1.10†	0.38
Treated with a low phenylalanine diet	17 days-8 years	27	77	0.89†	0.32

*Method of S. Udenfriend and J. R. Cooper, *J. Biol. Chem.*, 196: 227, 1952.
†Means significantly different ($p<0.01$) from normal controls and from each other.

43. INTRAVENOUS SODIUM-CHLORIDE-LOADING IN HYPERTENSION

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The therapeutic use of sodium-free diets in hypertension and epidemiological studies of sodium intake have focused attention on the role of the sodium cation in the pathogenesis of high blood pressure. Several investigators have employed the technique of recording the excretion of sodium in the urine after an intravenous load of sodium chloride. We have used the method of Hoobler *et al.* and have given 500 ml. of 2.5% sodium chloride per 1.73 sq. m. intravenously in 79 subjects. Fifty-three of these were hypertensive, of whom 30 were men and 23 were women; the rest were normal subjects. The hypertensive group included two cases of primary aldosteronism, two cases of pheochromocytoma, four cases of Goldblatt hypertension and three cases of Cushing's syndrome. These were studied preoperatively and postoperatively. One case of advanced chronic renal disease and one patient with the nephrotic syndrome were also included.

A correlation was found between the amount of sodium excreted in the first 1½ hours following the load and the level of diastolic blood pressure. A correlation also existed between the amount of sodium excreted following the load and the urine output. There was no similar correlation between potassium, urine flow and the diastolic blood pressure.

44. FURTHER EVIDENCE OF A COMMON RENAL TUBULAR TRANSPORT PATHWAY FOR POTASSIUM AND HYDROGEN IONS

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Clearance and excretory studies have suggested that potassium and hydrogen ions have a common transport pathway in the renal tubule and are secreted in a process of cationic exchange for sodium. Although stop-flow and micropuncture experiments suggest that this pathway is probably located in the distal tubule, an ionic competition for transport has not been demonstrated at this site. Potassium transport in dogs during respiratory acidosis was compared to that during respiratory alkalosis by the stop-flow method of Malvin *et al.* It is known that lowering pCO_2 will reduce tubular H^+ secretion and raising pCO_2 will have the reverse effect. Following respiratory acidosis, lowering pCO_2 produced a marked increase in potassium concentration in the distal area. $U/P_K/U/P_{INULIN}$ rose from 1.8 to 2.5, which suggested net potassium secretion. This was confirmed by clearances from free-flow periods. Conversely, maximal distal potassium concentration, induced by potassium infusion and a low pCO_2, was greatly decreased by elevation of the pCO_2. In addition to changes at the distal site, respiratory alkalosis produced a significant elevation of potassium concentration and $U/P_K/U/P_{INULIN}$ in more proximal areas. The present data are further evidence in support of a common tubular transport pathway for K^+ and H^+. It suggests competition between the two ions for this pathway, which appears to be mainly in the distal tubule. A less marked but still significant common pathway appeared to exist in more proximal areas of the nephron. This observation is compatible with recent micropuncture studies which show H^+ transport in the proximal tubule.

45. ACUTE ALTERATIONS OF PLASMA PROTEIN METABOLISM FOLLOWING THERAPEUTIC IRRADIATION

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In the course of studies of the acute radiation syndrome in man, tracer plasma protein turnover studies were carried out. Specific activity was measured in terms of the radioactivity of injected RISA per g. of total plasma protein. Irradiation of part of the trunk, or of the entire body, was carried out in a single dose after equilibration of the injected tracer. It was found that a rapid but transient decrease of 2-3% in the specific activity, measured as above, occurred about one to two hours after irradiation of part of the trunk, while, by contrast, a tendency for the specific activity to show a plateau occurred for some hours after irradiation of the entire body.

It is felt that these changes in specific activity are related causally to irradiation, since the greatest change, in those showing a transient decrease, coincided closely with the actual onset of radiation sickness, or with the expected time of sickness had the dose been slightly higher. In the group undergoing irradiation of the whole body, the end of the plateau occurred in closer relation to the cessation of clinical symptoms of acute radiation sickness.

In a few cases the specific activity of a crude albumin was determined also, and these, with additional more fragmentary data, are presented in discussion of the (as yet unresolved) part played by entry into the circulation of new protein and/or capillary and cell permeability changes and altered plasma protein dynamics in the few hours following substantial irradiation of a large volume of tissue.

46. RENAL TUBULAR TRANSPORT AND BONE METABOLISM IN A RARE FORM OF HYPOPHOSPHATEMIC RICKETS

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Normal bone structure requires integrity and equilibrium of organic and mineral phases. Availability of mineral for bone crystal formation may be altered at local or distant sites. In the latter case, where dietary vitamin D and mineral intake are normal, disturbed access or exaggerated rejection of mineral in the intestine and/or renal tubule may lead to rickets or osteomalacia.

We studied the above relationship in a rare form of hypophosphatemic rickets, associated with renal glucosuria and hyperglycinuria, occurring in an adolescent.

The mechanisms of the major biochemical features were investigated:

1. Glucosuria was identified chromatographically and shown to be dependent on defective tubular reabsorption.

2. Plasma glycine concentration was normal but tubular reabsorption was depressed. Tubular reabsorption of other aminoacids was normal.

3. Tubular reabsorption of inorganic phosphate (TRP) was only 60% of normal, when serum phosphorus averaged 1.0 mg./100 ml. Sustained hypercalcemia, induced by intravenous infusion, failed to improve the % TRP significantly.

These findings implicated a complex disturbance of renal tubular transport; renal biopsy revealed no structural lesion by light microscopy.

It was therefore implied that the rachitic lesion was secondary to tubular rejection of phosphate. As anticipated, detectable mineralization with the expected biochemical changes occurred after seven days of intravenous phosphate infusion. This result suggested that bone collagen was normal, a supposition supported by collagen composition analysis of a bone biopsy specimen. Histologically, osteomalacia was the only osseous abnormality identified.

Mineral balance studies revealed negative calcium balance (—200 mg./day) and negative phosphorus balance (—100 mg./day). Supplementary oral phosphate intake (approximately 2 g./day) was essential for healing, whereas vitamin D alone failed to correct plasma mineral or bone abnormalities.

The etiology of the renal transport lesion was apparently not hereditary, but an acquired cause could not be identified.

47. STUDIES ON THE RELATION BETWEEN THE KIDNEY AND ADRENAL STEROID SECRETION IN THE RAT

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Hypertension was produced in rats by the application of a silver clip to a renal artery. Aldosterone and corticosterone secretion rates were measured in the adrenal vein blood of these animals at varying times after the onset of hypertension, as measured by a tail plethysmograph. An increase in aldosterone secretion rate but not in corticosterone was observed in hypertensive animals studied from six to 12 weeks after the onset of hypertension. No significant effect on the hormone secretion rate could be demonstrated when animals were studied three, five or seven days after the application of a renal artery clip. Blood pressure measurements were not made on these animals, but more recent studies indicate that it is unlikely that an elevation in blood pressure was produced in this period. Nor could any effect on aldosterone secretion rate be demonstrated in hypophysectomized animals when a renal artery clip was applied immediately before collection of adrenal vein blood.

The effect of intravenous infusions of angiotensin II on the adrenal hormone secretion rate of hypophysectomized rats was also studied. Intrajugular infusions of angiotensin at the rate of 0.9 and 0.05 mg./minute resulted in increases in aldosterone secretion rate as well as measurable changes in the blood pressure as recorded from the carotid artery. Neither the blood pressure nor the aldosterone secretion rate was affected by infusions of angiotensin at the rate of 0.01 mg./min. Results on intermediate levels are reported. Preliminary results suggest that corticosterone secretion rates were not significantly increased at angiotensin infusion rates of 0.1 and 0.05 mg./min.

48. *IN VITRO* INCORPORATION OF THYMIDINE BY THE CELLS OF HUMAN BONE MARROW ASPIRATES

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An *in vitro* method for the detection of H_3-thymidine incorporation into the cells of human bone marrow aspirates was described at the annual meeting of the society in 1960. Further continuing studies have shown that:

1. The number of labelled cells in a series of aliquots from the same marrow collection can be reproduced with a standard deviation of ± 0.81%.
2. The presence of nutrient medium is not necessary for preservation of the ability of cells in a bone marrow aspirate to incorporate H_3-thymidine for a period of three hours during which the aspirate is maintained in a melting ice bath.
3. A progressive decrease in the number of cells capable of H_3-thymidine incorporation occurs with increasing time of exposure to glycerol.

Efforts to quantitate the effects of freezing and melting on H_3-thymidine incorporation into marrow elements have not been successful. It was found that elements which can incorporate H_3-thymidine are present in marrow aspirates after storage in the frozen state for periods up to 217 days.

49. THE ANTEPARTUM DIAGNOSIS OF HEMOLYTIC DISEASE BY ANALYSIS OF BILIRUBIN IN AMNIOTIC FLUID

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The bilirubin content of amniotic fluid samples obtained from 53 maternity patients at the time of rupture of the membranes was correlated with the clinical condition of the newborn infant. The bilirubin content was estimated by a modification of the method of Brückner, which is capable of estimating concentrations as low as 0.010 mg. %. Thirty-five of the infants were normal, 10 were alive with hemolytic disease, four were hydropic stillbirths and four were anencephalic stillbirths.

An amniotic fluid bilirubin content of less than 0.0353 mg. % was associated with 30 normal infants and one case of mild ABO hemolytic disease. An amniotic fluid bilirubin content over 0.057 mg. % was associated with 15 abnormal infants. There were seven fluids with bilirubin contents between 0.035 and 0.057 mg. %; five in normal infants and two in infants with hemolytic disease. It is concluded that estimation of the amniotic fluid bilirubin content alone is not a suitable basis for forecasting the health of the newborn infant if the content is in the range 0.035-0.057 mg. %. However, when the maternal antibody titre was positive, an amniotic fluid bilirubin content above 0.035 mg. % was always accompanied by an infant affected with hemolytic disease, whereas below this figure the infant was unaffected. It appears that a reliable forecast may be made when both the amniotic fluid bilirubin

content and the maternal antibody titre are considered.

50. ADRENERGIC BLOCKADE IN CLINICAL SHOCK

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A blocking dose of phenoxybenzamine (1 mg./kg.) was administered to each of 65 patients with clinical shock of varying etiology who had failed to show a satisfactory response to more conventional therapeutic measures. Prior to blockade all subjects showed clear-cut evidence of impaired peripheral circulation and urine flow rates of less than 20 ml. per hour. In spite of the presence of clinical evidence suggestive of adequate and in some cases overzealous blood volume replacement, 52 of the 65 subjects required additional fluid following adrenergic blockade in amounts ranging up to 3 litres in the four-hour post-block period. A prompt response to blockade—that is, amelioration of the clinical evidence of shock and coincident progressive rise in urine flow rate—occurred in 31 of the 65 patients. Improvement in peripheral circulation with blockade but with persistence of oliguria and subsequent development of acute renal failure occurred in 19 of the 65 subjects. In addition, one subject who showed an initial increase in urine flow rate with blockade later developed acute renal failure. Death occurred in 36 of the 65 subjects. In 12 this was attributable to irreversible shock, in 11 to the sequelae of acute renal failure and in the remainder to late complications of the initial shock-producing episode. The findings of this study lend support to the view that sympathetic nervous system overactivity is a frequent component of the picture of clinical shock and that measures designed to promote vasodilation may increase the chances of survival.

51. THE EFFECTS OF VARYING EXPERIMENTAL CONDITIONS ON SERUM BINDING OF THYROXINE AND TRIIODOTHYRONINE

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The binding of I^{131}-labelled thyroxine (T4) and triiodothyronine (T3) to the serum was studied, using starch gel electrophoresis as a means of separating the proteins. The gels were sectioned, and the amount of radioactivity associated with each protein area was measured by serial counting. Trace amounts of T4 (less than 0.5 μg./100 ml. plasma) became bound to certain plasma proteins in the following distribution: thyroxine-binding globulin (TBG) 40%, albumin 45%, and thyroxine-binding pre-albumin (TBPA) 15%. Triiodothyronine became bound to TBG (40%) and to the alpha and beta globulins in a non-specific manner. TBG was the only common binding protein for both hormones.

The effect of varying the pH of the gel from 7.0-8.8 was studied. The per cent of radioactive T4 associated with TBG and TBPA did not change appreciably from pH 7.2-8.4 but at higher pH values there was an increased TBPA and a decreased TBG binding. Decreasing the pH to 7.2 grossly changed the T3 binding, removing it completely from TBG to the non-specific alpha and beta globulins. This effect of pH on T3 binding explains the discrepancies in erythrocyte and resin uptake tests, using T3 as an indirect measure of T4 binding.

Loading experiments were carried out to saturate the TBG binding sites and to give a TBG capacity for the serum. Thus it was possible to study T3 binding and T4 binding at trace and saturated levels in serum under identical conditions in the gel.

52. ABNORMAL IODINE METABOLISM IN TWO PATIENTS WITH THYROID NODULES

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In recent years reports have appeared from different groups of workers on the production of abnormal iodinated compounds by the abnormal thyroid gland. In our laboratory, studies have been progressing on stable iodine metabolism, by the method of Mandle and Block (*Arch. Biochem. Biophys.*, 81:25, 1959), on thyroid tissue from several patients obtained by thyroidectomy or by biopsy. Among these, two patients were studied who were of unusual interest. Both were euthyroid, with a nodular goitre, confirmed histologically. Nodular tissues obtained at operation were enzymatically digested and the iodinated compounds separated by paper chromatography. In addition to iodotyrosines and free iodide, one of the patients showed the production of tetraiodothyroacetic acid (Tetrac) by the thyroid nodule. Production of tetraiodothyroacetic acid has been reported in tissues of experimental animals but this appears to be the first report of the formation of this substance in human thyroid tissue. The second patient showed the formation of an abnormal iodocompound, which was neither thyroxine nor triiodothyronine nor the acetic acid analogues of the thyronines. Efforts are being made to identify the compound. It would appear from these and other studies that there is a wide number of different abnormal metabolic pathways leading to the synthesis of abnormal iodinated compounds in various non-toxic goitres. The mechanisms involved, however, are not clear since little is known of the various enzymes that mediate in the biosynthesis of thyroid hormones.